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THE PREDOMINANCE OF CLONES IN POPULATIONS OF THE SEA ANEMONE *ANTHOPLEURA ASIATICA* (UCHIDA)

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ABSTRACT

To assess the population structure of the sea anemone *Anthopleura asiatica,* sex ratios and acrorhagial responses were examined among ten populations in the Seto Inland Sea of Japan. In spite of the fact that all specimens were capable of eliciting an acrorhagial response on contact with other individuals of the same species, no response was found in certain populations. These populations were always composed of individuals of the same sex. This result indicates that examination of the response was effective in distinguishing clones of the anemone. On the basis of sex and acrorhagial response, each of the populations seems to consist of a single or a few clones, and moreover, one clone appears to be distributed over 5 km through six separate locations. Diversity of clones among locations may imply sexual recruitment in the past. The anemones probably prosper asexually in local populations while dispersal to new habitats is facilitated by sexual reproduction. The causes of the clones' predominance and the applicability of the Strawberry-Coral Model to the anemone are discussed.

INTRODUCTION

Some sea anemones can reproduce in a variety of ways both sexually and asexually (Chia, 1976). It is interesting to study how these anemones use their sexual and asexual reproductive modes in their life cycles, because such studies may provide useful information which helps to answer the questions about the roles of sex (Williams, 1975). Genotypic structure of a population is a consequence of reproductive modes. However, few studies have examined sea anemone population structure: *Anthopleura elegantissima* (Francis, 1973a; Sebens, 1982), *Haliplanella luciae* (Shick, 1976; Shick and Lamb, 1977), *Metridium senile* (Hoffmann, 1976; Shick *et al.,* 1979; Bucklin and Hedgecock, 1982) and *Actinia tenebrosa* (Black and Johnson, 1979; Ayre, 1983, 1984).

Aggressive behavior using acrorhagi borne under tentacles has been known in sea anemones for some time (Abel, 1954; Bonnin, 1964; Francis, 1973b; Bigger, 1976, 1980; Brace and Pavey, 1978; Ottaway, 1978; Williams, 1978; Ayre, 1982). Acrorhagial responses are elicited by contact with other anemones of the same species and with other cnidarian species. On the other hand, it is known that no response occurs among clonemates, which have the same genotype. Therefore, the response between anemones must be useful to recognize clones and to assess the structure of populations (Sebens, 1982), although the usefulness of this distinguishing method may be limited (Bigger, 1980; Ayre, 1982).

Anthopleura asiatica, a common intertidal sea anemone in western Japan, repro-

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duces asexually by longitudinal fission, as well as sexually (Uchida, 1958, 1965). On the rocky shore of Matsugahana Pt. near Mukaishima Marine Biological Station, Japan, the anemones live in tidepools with their pedal discs in contact with each other (Fujii, 1985). When I examined anemone gonads, in 1983, to assess the reproductive cycle of anemones inhabiting the rocky shore, the noticeably mature specimens were always males (Fujii, in prep.).

The present study examines the sex ratio and acrorhagial responses of *A. asiatica* specimens at ten locations in the central Seto Inland Sea of Japan (Table I, Fig. 1), in order to assess the population structure of the anemone. The effectiveness of the acrorhagial response in distinguishing clones, the population structure, and the applicability of the Strawberry-Coral Model (Williams, 1975) to the species, are discussed on the basis of these results.

MATERIALS AND METHODS

Between August and September 1983, collections of *Anthopleura asiatica* individuals were made from ten locations which differed in size and habitat type: Kamiebujima Islet, Matsugahana Pt., Kannonzaki Pt., Sasajima Islet, Shijushima Islet, Iwashijima Is., Ategijima Islet, Toyoshima Is., Takaikamishima Is., and Aoshima Is. (Fig. 1, Table I). Although I also visited other locations on these and other islands, *A. asiatica* was only found at these ten locations. At three of them, Kannonzaki Pt., Toyoshima Is., and Aoshima Is., the anemones were found only in one or a few tidepools, while at the others, they were distributed over more than ten meters of shoreline. The anemones often form aggregations of contiguous individuals. In each location, the specimens were collected randomly from several aggregations which had been chosen throughout the habitats. The specimens from each aggregation were transported separately to the laboratory in labeled plastic bags and kept in seawater in labeled plastic cups (8 cm in basal diameter, 5 cm in depth) until they were examined. The seawater was changed daily.

Acrorhagial responses were examined using the specimens of the three aggregations which had been furthest away from each other in each location. Each anemone was allowed to attach to a piece of oyster shell in the cup, so that it could be moved easily. After two days, firmly attached anemones were paired with the specimens from the other two aggregations at the same location. A 1-second touch technique (Bigger, 1980) was applied to test the response. The pair's tentacles were lightly touched to each other for one second every fifteen seconds. Successive contacts were made ten times between the same tentacle tips, or until an acrorhagial response occurred. Over three repetitions were done in every three combinations. If no response was elicited in a pair, the pair was placed with their tentacles in contact and observed for more than one hour.

The acrorhagial response also was examined between anemones from different locations, using specimens from the three aggregations. Twenty-nine combinations were tested with at least five repetitions of each, using different pairs (Fig. 3).

Collected anemones, including those used for the response test, were all anaesthetized in the cups with a 50:50 solution of seawater and 7.5% (w/v) magnesium chloride. Well-relaxed anemones were cut longitudinally and their gonads were examined under a stereomicroscope.

In November 1983, another collection of anemones was made from five of the ten locations, Kamiebujima Islet, Matsugahana Pt., Sasajima Islet, Shijushima Islet, and Ategijima Islet, in order to examine the acrorhagial responses again. Specimens were collected from 6, 20, 13, 5, and 9 aggregations, respectively (five specimens at

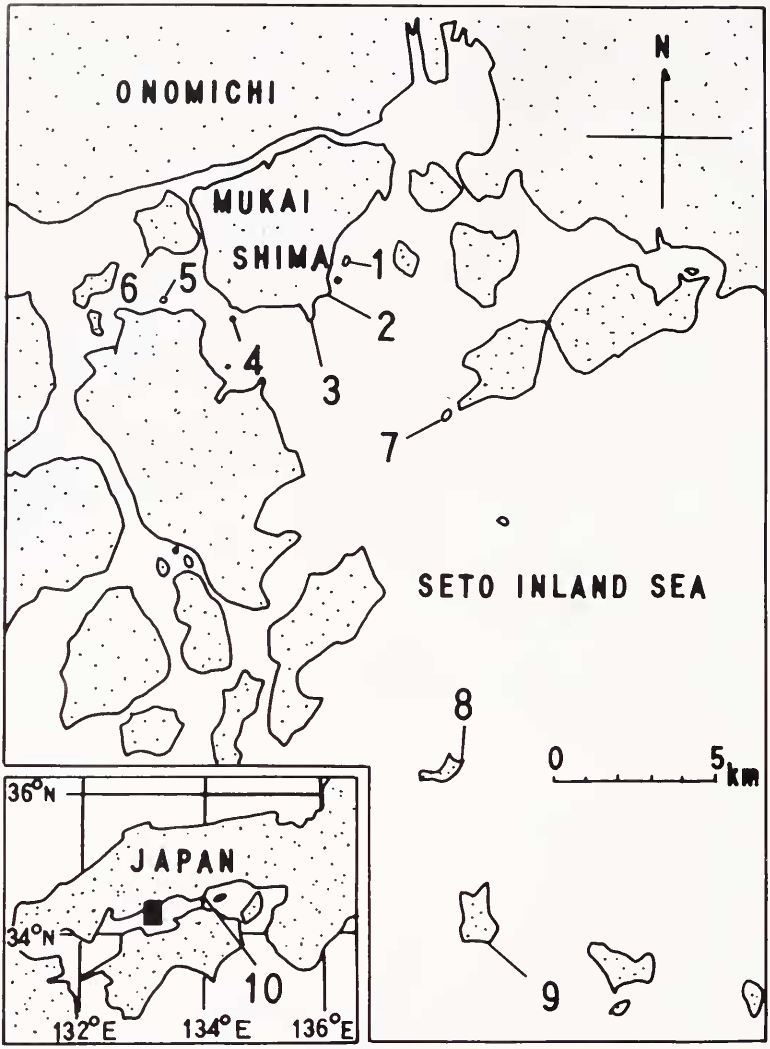

FIGURE 1. The map of the ten locations.

each aggregation). The responses were examined with every possible combination of the aggregations in each location and those of the location pairing their representatives. If no response occurred between their representatives, the rest of the specimens from different locations were set in contact with each other in a vessel and observed to confirm the results.

RESULTS

The sex of 408 specimens of *A. asiatica* was determined from 62 aggregations and they were found to be dioecious: 76 females, 281 males, 51 immature (Table II). Ripe individuals were easily sexed by the color and form of the gonads. Females' gonads

TABLE I

Description of locations

Locations	Ranges (area or length)	Habitat types
(1) Kamiebujima Islet	20 m	$t = p = r$
(2) Matsugahana Pt.	2000 m^2	$t \gg r > p$
(3) Kannonzaki Pt.	0.35 m^2	t
(4) Sasajima Islet	50 m	$r \gg p$
(5) Shijushima Islet	12 m	$p > t$
(6) Iwashijima Is.	* 22 m	t
(7) Ategijima Islet	40 m	$p \gg r = t$
(8) Toyoshima Is.	* 9 m^2	t
(9) Takaikamishima Is.	* 250 m	$p \gg r = t$
(10) Aoshima Is.	6 m^2	t

Relative abundance of inhabited habitants are represented by inequality signs. t: tidepool, p: pebble, r: rock. *After this study, the anemones were found at the next shores outside the reported ranges.

were filled with light blue eggs, and the white vesicles in the males were filled with sperm.

The sex ratios in most locations were extremely skewd (Table II). Only a single sex was found in eight of the ten locations. All specimens from six locations, Kamiebujima Islet, Mastugahana Is., Kannonzaki Pt., Sasajima Islet, Shijushims Islet, and Ategijima Islet, were males, while all specimens from two locations, Toyoshima Is. and Aoshima Is., were females. Although both sexes were found in the other two locations, Iwashijima Is. and Takaikamishima Is., even there, the specimens from an aggregation were always of one sex (Table II).

No acrorhagial responses were elicited in some combinations of the specimens; their tentacles were interlaced for over one hour without any response. But all anemones used in this study apparently had the potential to display the acrorhagial response because they always elicited the responses after several artificial tentacle contacts (from one to four times) in the other combinations. The process of the response in *A. asiatica* was similar to those in the other acrorhagi-bearing anemones (Bonnin, 1964; Francis, 1973b; Bigger, 1980): (1) tentacle withdrawing after contact; (2) expansion of acrorhagi; (3) leaning to the opponent; (4) application of acrorhagial ectoderm; and (5) recovery.

In each of the eight locations where all specimens were the same sex, no acrorhagial response was found in any pair of the three aggregations (Fig. 2). Additional examinations of acrorhagial response were carried out in five of the eight locations, combining more aggregations, but no response occurred in any of them (Table III). In fact, the responses were always found in two of the three combinations in the two locations where there were both males and females (Fig. 2), but even there, the specimens of the two aggregations which did not respond to each other were always the same sex.

As for acrorhagial responses between anemones from different locations, twenty-nine combinations were examined (Fig. 3). In this test the responses between males (Ategijima Islet *vs.* other male locations) and those between females (Aoshima Is. *vs.* Iwashijima Is.) were also observed. The acrorhagial response between anemones seems to be independent of their sex. The responses were elicited in every combination of the locations except those of the locations along Mukaishima Is., where all

TABLE II

Distributions of sexes in the ten locations

		Aggregations																
Locations		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	Total	
(1) Kamiebujima	M	5	5	3	5	5	10	5	5								M	43
	F	0	0	0	0	0	0	0	0								F	0
	I	0	0	2	0	0	0	2	0								I	4
(2) Matsugahana	M	6	5	4	5	5	5	5	5	5	5	5	4	5	25	9	M	98
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F	0
	I	0	0	1	0	0	0	0	0	0	0	0	1	0	8	27	I	37
(3) Kannonzaki	M	4															M	4
	F	0															F	0
	I	2															I	2
(4) Sasajima	M	5	5	5	5	5											M	25
	F	0	0	0	0	0											F	0
	I	0	0	0	0	0											I	0
(5) Shijushima	M	7	7	7	7	7	9										M	44
	F	0	0	0	0	0	0										F	0
	I	0	0	0	0	2	1										I	3
(6) Iwashijima	M	0	4	5	3												M	12
	F	8	0	0	0												F	8
	I	0	0	0	0												I	0
(7) Ategijima	M	8	11	6	5	5											M	30
	F	0	0	0	0	0											F	0
	I	0	0	0	0	0											I	0
(8) Toyoshima	M	0	0	0	0	0											M	0
	F	4	5	5	5	2											F	21
	I	1	0	0	0	3											I	4
(9) Takaikamishima	M	5	5	5	0	0	0	0	5	5							M	25
	F	0	0	0	5	5	5	5	0	0							F	20
	I	0	0	0	0	0	0	0	0	0							I	0
(10) Aoshima	M	0	0	0	0												M	0
	F	5	5	5	12												F	27
	I	0	0	1	0												I	1

M: male, F: female, I: immature.

specimens were males. In order to make sure of the results, particularly the exception, additional examinations were done combining five locations; four of them along Mukaishima Is. No response was also found among the four locations while the responses were always elicited in combinations between the fifth location, Ategijima Islet, and one of the other four (Table III).

DISCUSSION

Sea anemones bearing acrorhagi display aggressive behavior on contact with the same species or with other cnidarian species (Bigger, 1980). This study shows that *A. asiatica* responds aggressively to other individuals of the species with the same pattern of behavior as other acrorhagus-bearing anemones (Abel, 1954; Bonnin, 1964; Francis, 1973b; Bigger, 1976, 1980; Ottaway, 1978; Williams, 1978; Ayre, 1982).

Acrorhagial response seems to be useful to the assessment of the genetical struc-

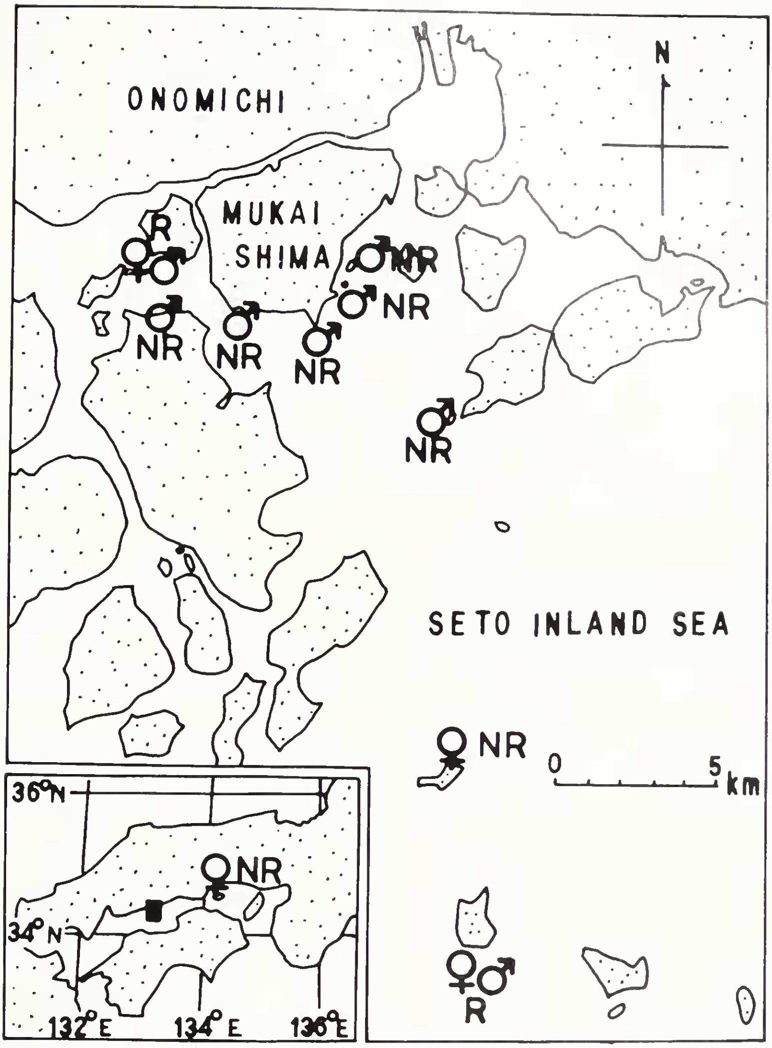

FIGURE 2. Acrorhagial responses among three aggregations in each location. R: the responses occurred in two of three combinations of aggregations. NR: no response was elicited. Signs of sexes represent the sex compositions of the locations.

ture of anemone populations because anemones are able to distinguish between clonemates and non-clonemates (Francis, 1973a, b, 1976; Bigger, 1976, 1980; Ayre, 1982), although their distinguishing ability may be limited. Ayre (1982) failed to produce aggressive responses on contact between some genetically dissimilar individuals of *Actinia tenebrosa*. When we use the acrorhagial response to distinguish clones, it is important to know to what extent we can trust the response.

A clone of dioecious animals is composed of a single sex. Therefore distribution of the sexes can be a useful indication of asexual reproduction, especially in sessile animals such as anemones (Ford, 1964; Sebens, 1982). The exceedingly skewed sex ratios in the locations (Table II) suggest that the anemones reproduce asexually there.

TABLE III

Results of the second acrorhagial response test within and between five locations

	(1)	(2)	(4)	(5)	(7)
(1) Kamiebujima Is.	– C(6.2)	–	–	–	+
(2) Matsugahana Pt.		– C(18.2)	–	–	+
(4) Sasajima Islet			– C(13.2)	–	+
(5) Shijushima Islet				– C(5.2)	+
(7) Ategijima Islet					– C(9.2)

–: no responses were elicited in the combination, +: the responses were always elicited. The number of tested combinations within locations were represented by C(N.2) = (N!/(N − 2)!)/2, where N is the number of collected aggregations. As for the test between locations, at least five repetitions were done. All specimens of (1)–(5) were mingled in a vessel without any response.

The acrorhagial responses of anemones are thought to be independent of their sexes (Ayre, 1982; Sebens, 1984). In this study, *A. asiatica* responded aggressively to both male and female specimens from different clones. Therefore, assuming the test of the response was ineffective in distinguishing clones, one would expect to find individuals of both sexes among the anemones that did not respond to each other. However, anemones that did not respond were always of the same sex (Fig. 2, 3). This indicates that *A. asiatica*'s ability to distinguish clones is very good.

Asexual reproduction has a great influence on genetical structure of a population. Sea anemones which reproduce asexually have less genotypic diversity in a population than would be expected in a sexual population with free recombination and random matings (Shick and Lamb, 1977; Black and Johnson, 1979; Ayre, 1983; Hoffmann, 1986; Shick and Dowse, 1985). The results of this study indicate that the populations of *A. asiatica* have extremely low diversities. Eight of the ten locations seem to consist of single clones, although some minor clones may have been missed. Even the other two locations seem to consist of a few dominant clones. Moreover, this study suggests that one of the clones may dominate at six locations, covering a range of over five kilometers (Fig. 3). This might be explained by a tidal current passing through the locations, but more evidence will be needed to substantiate this idea. It has been reported that the sea anemones, *A. elegantissima* and *Metridium senile,* form clonal aggregations (Ford, 1964; Francis, 1973; Hoffmann, 1976; Sebens, 1982) and a clone of them can be distributed over a relatively large area in their populations (Shick *et al.,* 1979; Sebens, 1982). It has also been shown that the populations of the sea anemone *H. luciae,* introduced on the Atlantic coast of North America, are composed of one or a few clones (Shick, 1976; Shick and Lamb, 1977; Shick *et al.,* 1979). However, the formation of discrete colonies by a single clone had not been reported until now.

As Shick *et al.* (1979) suggested for *H. luciae,* single clone predominances in *A. asiatica* may be caused by one or more of the following: (1) the clone best adapted to the habitats was selected; (2) there were few immigrants and new settlements so that the few lucky anemones arriving on unoccupied habitats could prosper and predominate there, or (3) each clone at a location had excluded others aggressively. Ayre (1985) revealed through a field transplantation experiment that populations of *A.*

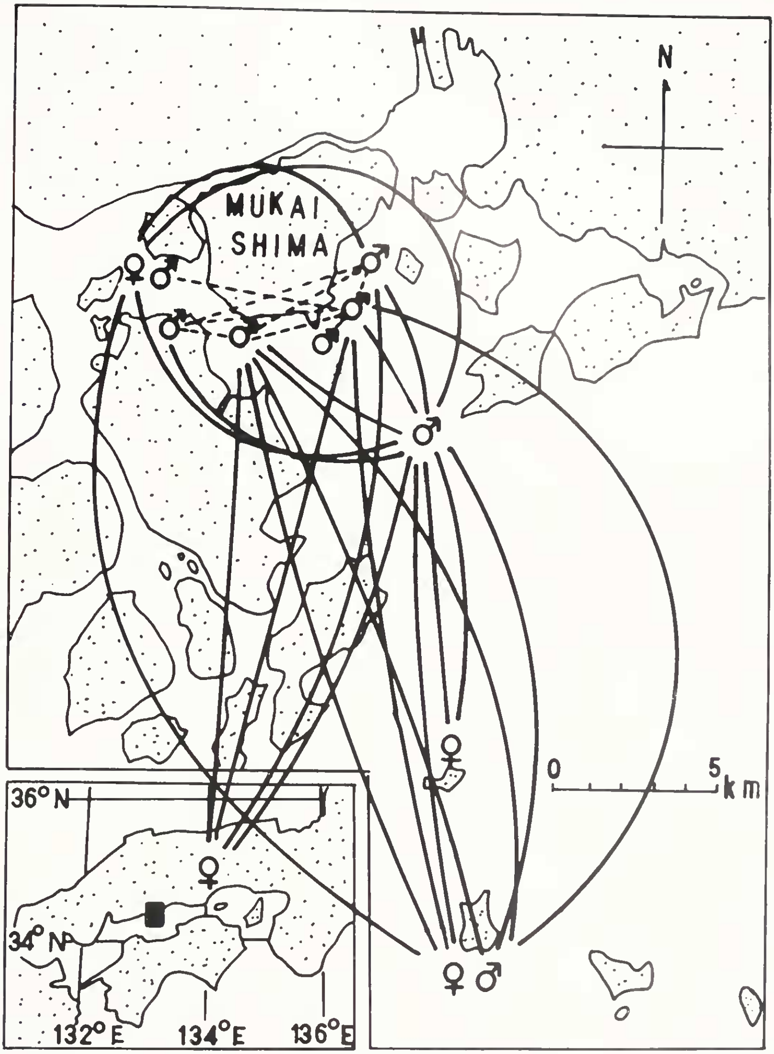

FIGURE 3. Acrorhagial responses among locations. No response occurred between the locations connected by broken lines. The responses occurred between the locations connected by solid lines.

tenebrosa consist of highly locally adapted clones. The anemones showed higher asexual fecundity when they were transplanted into their original habitats. In the center of the Inland Sea, populations of *Anthopleura asiatica* seem to consist of one or a few clones. Even if the clones are the best adapted to their locations, they probably do not have narrow but rather wide adaptational ranges, because each location consists of a variety of habitat types (*e.g.,* tidepool, rock, and boulder at different tidal heights). The clones of the colonizing anemone, *H. luciae,* are adapted to a wide range of temperature and salinity (Shick, 1976; Shick and Lamb, 1977; Shick and Dowse, 1985). Shick *et al.* (1979) revealed that in *H. luciae,* the preadaptations of some clones to low temperature allowed their predominance. Although such a severe condition could not be found out in the Seto Inland Sea, there may be a different kind of limiting condition which functions as a bottleneck. A variety of clones would be expected in an unstable habitat (Sebens, 1982; Ayre, 1984). Because the predominance of a single or a few clones occurs even on the boulder beaches, which seem to be unstable, it also seems plausible that the predominant clones started as a few lucky founders which encountered unoccupied habitats. A low rate of new recruitment may allow the clones to prosper in diverse and unstable habitats. Aggressive interactions of anemone are thought to play an important role in intra-specific competition for space (Francis, 1973a, b Ayre, 1983). But it is still uncertain whether one clone can exclude others completely from its habitats through aggressive interactions. In fact, Bigger (1980) suggested that acrorhagial response can be viewed as one of a set of ecological facters which may maintain a genetic heterogeneity. In any case, they may serve to repel the other invading clones once a clone occupies a habitat (Sebens, 1982).

The present study suggests not only asexual proliferation of *A. asiatica* in the local populations but also their sexual settlement in the past. On the basis of the aggressive response, each location seems to be occupied by different clones, with the exception of the locations around Mukaishima Is. (Fig. 3). This diversity was probably brought about through sexual reproduction. *A. asiatica* seems to use sexual reproduction for wide dispersal while using asexual reproduction in local proliferation.

The life-history pattern of *A. asiatica* appears to fit the predictions of the Strawberry-Coral Model which Williams (1975) proposed to overcome the theoretical disadvantage of sex. The model assumes that clonal proliferation of sessile organisms is limited by their habitat space, and that there is an intense selection, so that only the progeny with sufficiently high local fitness can win in each habitat. In the model, diverse sexual progeny are predicted to have a greater probability of establishing new colonies outside the limits of original habitats, because of the different demands of new habitats.

In spite of the conformity in the life history pattern, some situations of *A. asiatica* may be inconsistent with the assumptions of the model. For the start, a clone of the species seems to inhabit a variety of habitat types. From this situation, it is likely that the clones were not the fittest but just the lucky ones (intense competition may be rare) and/or that each clone has a wide adaptational range (*i.e.,* variability may be less important). Thus the model may not be applicable in each case. Aggressiveness may be another problem (Ayre, 1983), because it does not seem to be dependent on environmental conditions. If the most aggressive clone was selected in every habitat, the introduction of variation through sexual reproduction might be disadvantageous.

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